Clinical relevance of IgA rheumatoid factor (RF) in children with juvenile rheumatoid arthritis

Abstract This study proposed to investigate the prevalence and clinical relevance of serum immunoglobulin A (IgA) rheumatoid factor (RF) in juvenile rheumatoid arthritis (JRA) as published reports vary in their conclusion. Sera of 82 children with JRA and 25-age and sex-matched healthy children were measured for IgA RF by an enzyme linked immunoassay using human IgG as the antigen. Forty-three percent of the disease population were positive and the prevalence in pauciarticular, polyarticular and systemic onset was 9/18 (50%), 21/47 (44.7%) and 5/17 (27.7%) respectively when mean + 2SD of normal was taken as the cut-off value. By defining the upper limit of normal as mean + 6SD, 16/47 (34%) were positive in the polyarticular as compared to 2/18 (11.1%) in pauciarticular and 1/17 (5.8%) of systemic onset disease groups. The prevalence in the polyarticular subset with the upper cut-off limit was significantly higher than the pauciarticular and the systemic onset group ($P<0.05$). Furthermore, the mean level of IgA RF was significantly higher in the polyarticular group compared to the mean level in the systemic onset group ($P<0.05$). The mean level of IgA RF was also significantly higher ($P<0.05$) in 61 children with active diseases.

Key words Childhood arthritis · Autoantibodies · Anti IgG

Introduction

The majority of children with juvenile rheumatoid arthritis (JRA) are negative for rheumatoid factor (RF) by latex agglutination whereas with the use of a sensitive assay, such as enzyme-linked immunosorbent assay (ELISA), 50–70% of these patients are positive for IgM RF [1]. Moreover, by using this technique it is possible to study IgG RF and IgA RF. This is relevant since IgA RF has been used as a marker of disease severity in adult patients with rheumatoid arthritis (RA) [2, 3, 4]. While the prevalence of IgA RF has been reported to vary from 22 to 58% in patients with JRA [5, 6, 7], the clinical relevance of this finding has not been consistent with one study [6] reporting IgA RF to be specifically present in active polyarticular disease and another [5] in the three subsets, namely polyarticular onset, pauciarticular onset and systemic onset.

We have previously reported a significant association of deforming joint disease with the presence of IgM RF [8]. Subsequently, we found an association of early onset pauciarticular disease with IgM rheumatoid factor that is complexed with IgG or hidden RF in our cohort of children with JRA [9]. In this study, we have tried to find the prevalence and clinical relevance of IgA RF in JRA.

Patients and methods

Eighty-two consecutive children fulfilling the American College of Rheumatology (ACR) criteria [10] of JRA and seen between 1989 and 1997 in the Clinical Immunology Department of old tertiary care referral hospital were included in the study. Clinical details of disease duration, morning stiffness, age, sex, subtypes, fever, rash, lymphadenopathy, number of active and swollen joints, and limitation of movement were recorded. The disease was categorised as active if there was presence of systemic symptoms (fever, rash, lymphadenopathy, hepatosplenomegaly) and/or tender swollen joint with raised erythrocyte sedimentation rate (ESR; $>30$ mm fall in 1st hour) and C-reactive protein (CRP; $>0.6$ mg/dl). Sera samples were collected and stored at $-40 \, ^\circ C$ until analysis.

ELISA for IgA RF

Necessary modifications were made in the Faith’s protocol for detection of IgM RF [11]. Ninety-six well flat-bottom microtitre plates (Nunc) were coated with 100 $\mu$l of 10 $\mu$g/ml of human IgG (Sigma, St. Louis, Mo., USA) in 0.05 M sodium carbonate buffer
(pH 9.6) for 2 h at 37 °C followed by overnight incubation at 4 °C. The plates were washed with phosphate-buffered saline (PBS; 0.15 M, pH 7.2) and blocked with 150 μl of PBS containing 3% bovine serum albumin (PBS-BSA) for 3 h at 37 °C. After washing three times with PBS containing 0.05% Tween 20 (PBS-T), 100 μl of 1:250 diluted serum in PBS–BSA was added to each well and incubated for 2 h at 37 °C. Following further three washings, 100 μl/well of 1:4000 diluted antihuman IgA HRP conjugate (DAKO, Denmark) was added and the plate was incubated for 2 h at 37 °C. The plate was then extensively washed with PBS-T and developed with 50 μl/well of orthophenyenediamine solution in citrate phosphate buffer containing 30% hydrogen peroxide. The reaction was stopped after 30 min by adding 25 μl/well of 4 N sulphuric acid. The absorbance was read at 492 nm using an ELISA reader.

Serum sample from a patient with RA containing IgA RF was used as standard in each plate. Doubling dilution of this serum yielded a sigmoid shaped curve. The lowest level of detection, i.e. where the curve flattened out was assigned as 1 arbitrary unit (au/ml). All the test and control sera were read against this curve. Mean (1.8 au/ml) + 2SD (1.18), i.e. 4.16 au/ml of 25 control sera was taken as the cut-off limit. For some analyses, sera having a value higher than the mean + 6SD (8.8 au/ml) were considered as positive.

### Statistical analysis

Student’s t-test and the Z-test for proportions were used for intergroup comparisons and the chi-squared test was used to test the difference in prevalence between males and females. The relationship of IgA RF with other quantifiable clinical parameters and IgM RF was analysed using Pearson’s correlation coefficient test.

### Results

Of the 82 patients, 47 had polyarticular, 18 pauciarticular and 17 systemic onset type of JRA. The mean age of patients was 13.9 years and the median duration of disease was 4 years (Table 1). Sixty-one children, 37 of polyarticular and 12 each of pauciarticular and systemic onset type had active disease at the time of inclusion in the study. Seven of the 47 children with polyarticular disease had classical RF as detected by latex agglutination.

Thirty five (42.7%) children were positive for IgA RF. The prevalence in three different types was: polyarticular, 21/47 (44.7%); pauciarticular, 9/18 (50%); and systemic onset, 5/17 (29.4%). This difference was not statistically significant. The majority of positive sera in each subset of disease had classical RF as detected by latex agglutination.

### Table 1 Clinical profile of juvenile rheumatoid arthritis (JRA) patients (Poly Polyarticular, Pauci Pauciarticular)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Poly</th>
<th>Pauci</th>
<th>Systemic</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>47</td>
<td>18</td>
<td>17</td>
<td>82</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>15.3</td>
<td>12.5</td>
<td>11.5</td>
<td>13.9</td>
</tr>
<tr>
<td>Range</td>
<td>5-35</td>
<td>6-23</td>
<td>4-18</td>
<td>4-35</td>
</tr>
<tr>
<td>Sex ratio (M:F)</td>
<td>21:26</td>
<td>14:4</td>
<td>12.5</td>
<td>47:35</td>
</tr>
<tr>
<td>Median disease</td>
<td>5</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>duration (years)</td>
<td>0.5–20</td>
<td>0.25–9</td>
<td>0.2–8</td>
<td>0.2–20</td>
</tr>
</tbody>
</table>

(Fig. 1). To avoid the blunting effect of the low positivity upon any true difference that may be there, the cut-off limit was set at a higher limit of mean + 6SD (8.8 au/ml). The seroprevalence in the polyarticular subset 16/47 (34%) was significantly (P < 0.05) higher than the pauciarticular (2/18; 11.1%) and the systemic onset group (1/17; 5.8%). The mean level of the IgA RF was also significantly (P < 0.05) higher in the polyarticular (9.6 + 11.5 au/ml) compared to the systemic onset (4.4 + 72 au/ml) but not with the pauciarticular subtype (5.8 + 7 au/ml).

IgA RF was present more often in females with polyarticular disease (16/26, 61.5%) than males (5/21, 23.8%; P < 0.05). Patients with active disease had a higher mean IgA RF (9.15 au/ml) than inactive disease (4.6 au/ml, P < 0.05). No correlation was seen between IgA RF and individual clinical and laboratory parameters of disease activity, such as tender joint count, duration of morning stiffness, haemoglobin (Hb), ESR and CRP. IgA RF had good correlation with IgM RF (r = 0.706, P < 0.05).

### Discussion

This study confirms that the level of IgA RF is higher in the polyarticular subset and serves to differentiate polyarticular disease from the other subtypes of JRA. The three subtypes of JRA have different clinical expressions and characteristic pathogenic mechanisms of each subset. In our continued effort to look for serological markers, which are distinctive of these subsets, we first looked for IgM RF [8] and subsequently hidden IgM RF [9]. IgM RF identified patients with deforming disease but its distribution was seen in all the three subsets of disease. Hidden IgM RF was also seen in all the three subsets though it was significantly associated with early onset pauciarticular disease. The prevalence of 42.7% patients is within the range of previous report.